Predictive value of Escherichia coli susceptibility in strains causing asymptomatic bacteriuria for women with recurrent symptomatic urinary tract infections receiving prophylaxis

Predictive value of *Escherichia coli* susceptibility in strains causing asymptomatic bacteriuria for women with recurrent symptomatic urinary tract infections receiving prophylaxis

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Abstract

A significant proportion of women develop a recurrence following an initial urinary tract infection (UTI). In women with recurrent UTI, the predictive value of asymptomatic bacteriuria (ASB) for the development of a subsequent UTI has not yet been established and it is not known whether information from an asymptomatic sample is useful in guiding antimicrobial therapy. To address these questions, we used data that originated from the 'Non-antibiotic prophylaxis for recurrent urinary tract infections' (NAPRUTI) study: two randomized controlled trials on the prevention of recurrent UTI in non-hospitalized premenopausal and postmenopausal women (n = 445). During 15 months of follow-up, no difference was observed in the time to a subsequent UTI between women with and without ASB at baseline (hazard ratio: 1.07, 95% CI 0.80–1.42). The antimicrobial susceptibility and pulsed-field gel-electrophoresis (PFGE) pattern of 50 *Escherichia coli* strains causing a UTI were compared with those of the ASB strain isolated 1 month previously. The predictive values of the susceptibility pattern of the ASB strain, based on resistance prevalence at baseline, were ≥76%, except in the case of nitrofurantoin- and amoxicillin-clavulanic acid-resistance. Asymptomatic and symptomatic isolates had similar PFGE patterns in 70% (35/50) of the patients. In the present study among women with recurrent UTI receiving prophylaxis, ASB was not predictive for the development of a UTI. However, the susceptibility pattern of *E. coli* strains isolated in the month before a symptomatic *E. coli* UTI can be used to make informed choices for empirical antibiotic treatment in this patient population.

Keywords: Antibiotic susceptibility, asymptomatic bacteriuria, *Escherichia coli*, pulsed-field gel-electrophoresis, recurrent urinary tract infection

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Introduction

Approximately 50% of all women will experience a urinary tract infection (UTI) during their lifetime [1] and 27 and 44% develop a recurrence following an initial UTI within 6 months and 1 year, respectively [2,3].

In women who have had at least three episodes of UTI in a period of 12 months, low-dose antibiotic prophylaxis has proved to be effective in reducing the number of recurrences [4]. However, this regimen has the disadvantage that it results in the development of antimicrobial resistance [5].

In addressing this antimicrobial resistance problem in women with recurrent UTI (rUTI), the information that can be derived from earlier periods of asymptomatic bacteriuria (ASB) has not yet been investigated. ASB is a condition in which uropathogens are isolated from a urinary sample while the patient is not experiencing symptoms indicating a UTI [6]. The relationship between ASB strains and strains that cause a subsequent UTI has been studied in several populations, but has not been addressed in women with rUTI

[7–10]. In this population the predictive value of ASB for the development of a subsequent UTI has not been established, nor has the question of whether the antibiotic susceptibility pattern of a microorganism isolated from an 'asymptomatic' sample can be used to determine the optimal empirical antimicrobial therapy for a subsequent UTI.

We have therefore investigated, in women with rUTI receiving either antibiotic or non-antibiotic prophylaxis, whether the presence of ASB predisposes to the development of a UTI. Furthermore, we determined whether the *Escherichia coli* ASB strain isolated in the month preceding an *E. coli* UTI was predictive for the strain causing the UTI, by comparing antimicrobial susceptibilities and pulsed-field gel-electrophoresis (PFGE) patterns of the bacteria isolated in both episodes.

Materials and Methods

Patients

We used data that originated from the 'Non-antibiotic prophylaxis for recurrent urinary tract infections' (NAPRUTI) study [5,11]. Briefly, this study consisted of two randomized controlled multicentre trials comparing 12 months of prophylaxis with cranberries (in premenopausal women) or lactobacilli (in postmenopausal women) with trimethoprim/sulfamethoxazole prophylaxis. Non-hospitalized women over 18 years who had experienced at least three symptomatic UTIs in the year preceding enrolment were eligible for inclusion. Patients were excluded when symptoms of UTI were noted at baseline. Recruitment took place from January 2005 to August 2007.

Midstream urinary samples were collected monthly and when symptoms of UTI were observed during 15 months of follow-up. Dipslides (Uriline, 56508, Biomérieux, Plainview, NY, USA) were prepared from all collected urinary samples and sent to the microbiological laboratory of Maastricht University Medical Centre for identification of the microorganisms and testing of the antimicrobial susceptibility.

If women had a history of functional or structural abnormalities of the urinary tract, of metabolic or hormonal abnormalities, or of impaired host responses, UTIs experienced during follow-up were classified as complicated. UTIs from all other women were classified as uncomplicated. This information was retrieved from the NAPRUTI baseline questionnaire, which also included questions about the number of UTIs in the year preceding enrolment and the sexual activity.

The study protocol was approved by the Medical Ethics Committees of all participating centres, and participants provided written informed consent before inclusion.

Predictive value of ASB for the development of UTI

To investigate whether the presence of ASB was predictive for the development of UTI, the participating women were divided into two groups, based on the presence of ASB at baseline. ASB was defined as having $\geq 10^5$ CFU/mL) on the baseline dipslide. A UTI was defined as the presence of self-reported symptoms of a UTI together with the isolation of a uropathogen ($\geq 10^3$ CFU/mL) [12]. Because not all patients had sent a urine sample when they had symptoms of a UTI, we used occurrence of a clinical recurrence as a secondary endpoint, which was defined as the presence of self-reported symptoms of UTI without microbiological confirmation [5].

Predictive value of ASB susceptibility and PFGE patterns

Patients were selected in whom the first UTI during the study was caused by *E. coli*. From this group (n = 160), the patients were identified in which *E. coli* was detected in the urine specimen collected in the month preceding the first *E. coli* UTI (UTI minus 1 sample, n = 76).

Using a random selection procedure, stratified for menopausal status and for complicated versus uncomplicated UTI, 50 patients were chosen for the assessment of the antimicrobial susceptibility and PFGE patterns of their *E. coli* isolates. A flow-chart of this selection procedure is given in Fig. 1.

Antimicrobial susceptibility testing

The microdilution method used for determining the antimicrobial susceptibility of the *E. coli* isolates involved Mueller–Hinton II cation-adjusted broth (Becton and Dickinson Company, Sparks, MD, USA), an inoculum of 5×10^5 CFU/mL and overnight incubation at 35°C. The MIC plates with freeze-dried antimicrobial agents were provided by MCS Diagnostics (Swalmen, the Netherlands). Methods and susceptibility breakpoints were in accordance with the EUCAST guidelines [13].

Pulsed-field gel-electrophoresis

PFGE was carried out using *Xba*I (New England Biolabs Inc., Beverley, MA, USA) and interpreted as previously described [14,15].

Statistical analysis

For the comparison of two groups a Pearson's chi-square test was used for categorical variables and a Student's t-test or Mann–Whitney U-test was used for continuous variables.

The cumulative probability of being UTI-free during the 15 months of follow-up for women with and without ASB at baseline was studied using Kaplan–Meier estimates, with the log rank test for the comparison. Subsequently, Cox regression analyses were conducted, which included the following

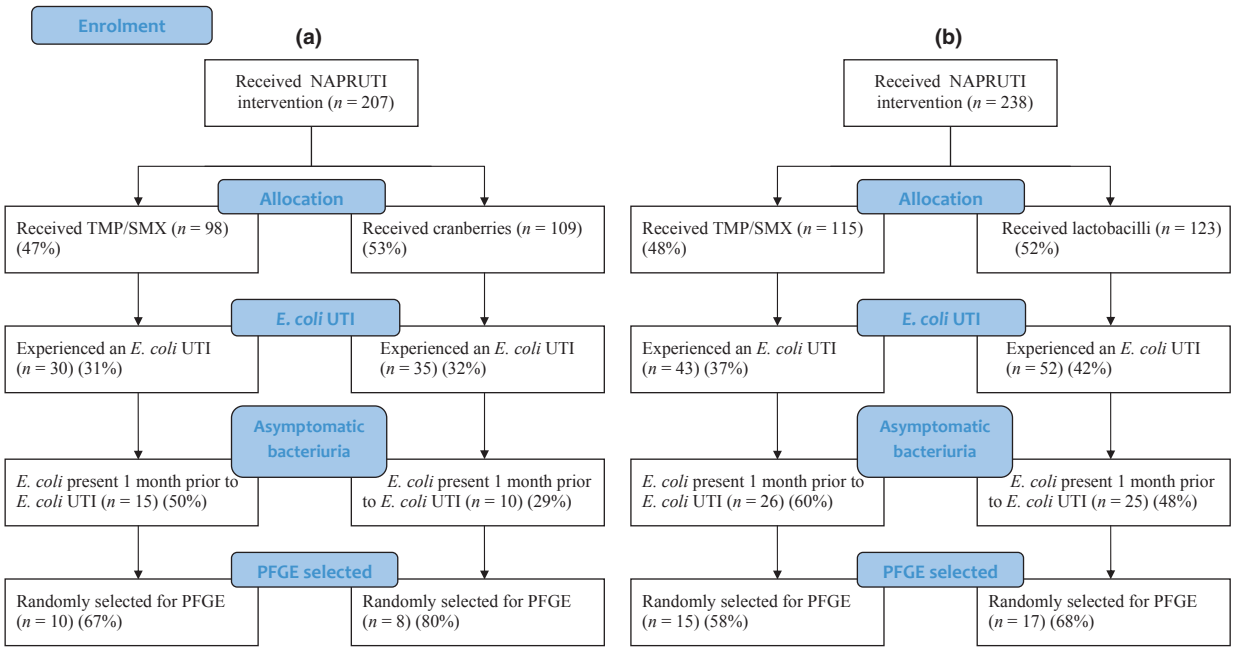

FIG. 1. Flow diagram showing the pulsed-field gel-electrophoresis (PFGE) and antimicrobial susceptibility selection procedure for premenopausal women (a) and postmenopausal women (b). TMP/SMX, trimethoprim/sulfamethoxazole.

potential confounders: age (in years), number of UTIs in the year preceding enrolment, sexual activity (yes/no), premenopausal status (yes/no), received trimethoprim/sulfamethoxazole prophylaxis (yes/no) and the presence of complicating host factors (yes/no).

To test for potential effect modification by menopausal status or received intervention or uncomplicated versus complicated UTI, interaction terms between these variables and presence of ASB at baseline were included in the statistical models. As these terms were not statistically significant, they were removed from the models and risk estimates were calculated for all women together.

In comparing susceptibilities per antimicrobial agent, asymptomatic and symptomatic strains were considered to be similar when they differed by no more than one dilution step. However, isolates were not considered similar when one of them was classified as 'susceptible' and the other as 'resistant'.

For each antimicrobial agent tested, positive and negative predictive values (PPV and NPV, respectively) were calculated as described by Vellinga *et al.* [16]. Calculated PPVs and NPVs are based on the prevalence of resistance of the *E. coli* strains isolated at baseline. To assess the influence of trimethoprim/sulfamethoxazole prophylaxis, we recalculated our PPVs and NPVs based on the prevalence of resistance of *E. coli* strains isolated after 12 months of trimethoprim/sulfamethoxazole prophylaxis. PPV was the proportion of patients who had an asymptomatic isolate resistant to an antibiotic, in whom the subsequent symptomatic isolate was also resistant to this antibiotic. NPV was the proportion of patients in whom the asymptomatic isolate was susceptible to an antibiotic and the symptomatic isolate also.

SPSS 16.0 was used for statistical analyses and $p < 0.05$ was considered statistically significant. For our power calculation we used the program STATA, version 12.0 [17,18].

Results

Patients and samples

A total of 207 premenopausal and 238 postmenopausal women participated. The number of ASB and UTI episodes experienced is given in Table 1.

Of 209 patients who experienced a UTI, 160 (77%) had *E. coli* as the causative uropathogen. One month preceding these *E. coli* UTIs, *E. coli* was isolated from 76 patients (48%). The mean time interval between the isolation of the included asymptomatic and subsequent symptomatic *E. coli* strains was 15 days (95% CI 12–17 days). These patients were stratified according to trial (premenopausal (A) versus postmenopausal (B)) and complicated (C) versus uncomplicated (U) status resulting in four groups, i.e. AC, AU, BC and BU. Four out of five (80%) AC, 14/20 (70%) AU, 16/26 (62%) BC and 16/25 (64%) BU patients were selected randomly for susceptibility and PGFE analysis (Fig. 1a,b).

TABLE 1. Asymptomatic bacteriuria and urinary tract infection episodes

	Premenopausal women (*n* = 207)	Postmenopausal women (*n* = 238)	Total study population (*n* = 445)
ASB at baseline[a]	62 (31)	113 (48)	175 (40)
ASB at any moment during study[b]	170 (82)	219 (92)	389 (87)
UTI[c] at any moment during study[b]	82 (40)	127 (53)	209 (47)

ASB, asymptomatic bacteriuria; UTI, urinary tract infection.
Numbers are *n* (%).
[a]Baseline dipslides were collected from 433/445 (97%) of the patients: 199/207 (96%) premenopausal and 234/238 (98%) postmenopausal women.
[b]Study duration was 15 months.
[c]Defined as the presence of self-reported symptoms of UTI in combination with the isolation of a uropathogen ($\geq 10^3$ CFU/mL).

Predictive value of ASB for the development of a UTI

When comparing the probability of being UTI-free during 15 months of follow-up between women with and without ASB at baseline, no difference was found (43% versus 45%, p >0.05) (Fig. 2). After inclusion of the confounding factors, ASB at baseline was still not predictive (hazard ratio 1.07, 95% CI 0.80–1.42). When clinical recurrence was used as the endpoint, outcomes changed only marginally (data not shown). Considering the number of participants for whom the ASB status was known at baseline (*n* = 433), the proportion of patients without ASB at baseline (60%, 258/433) and the probability of being UTI-free for women without ASB at baseline (45%), we would have been able to detect, at power >80% and $\alpha = 0.05$, a hazard ratio of 1.5 for developing a microbiologically confirmed UTI in the follow-up period.

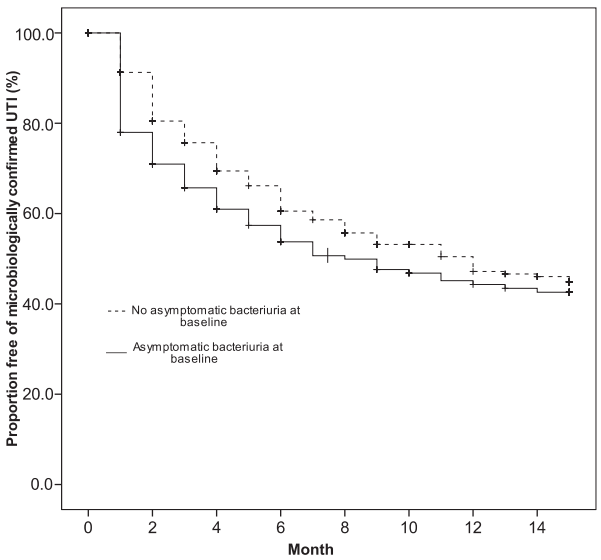

FIG. 2. Kaplan–Meier curves for the cumulative probability of being urinary tract infection (UTI) -free during the 15 months of follow-up, for women with and without asymptomatic bacteriuria at baseline. No difference was observed between the two groups (p >0.05).

Predictive value of ASB susceptibility and PFGE patterns

Table 2 gives PPVs and NPVs of the ASB strains. When PPVs and NPVs were based on the prevalence of resistance after 12 months of trimethoprim/sulfamethoxazole prophylaxis, the NPVs of amoxicillin (67%), trimethoprim (24%) and trimethoprim/sulfamethoxazole (24%) decreased because of the development of resistance to these agents. For amoxicillin-clavulanic acid, 7/13 (54%) discordant pairs differed by only one dilution step, but were classified as dissimilar because the asymptomatic *E. coli* was resistant whereas the symptomatic *E. coli* was susceptible. PFGE patterns were similar in six of these seven *E. coli* pairs (86%).

From the 50 randomly selected patients, PFGE-concordant *E. coli* isolates were observed in 35 women (70%). Stratification of results into the categories AC, AU, BC and BU showed that, in all groups, over 60% of the *E. coli* pairs were similar (75%, 79%, 63% and 69%, respectively, Fig. 3). Also, no difference was observed between the prophylactic regimens: 72% (trimethoprim/sulfamethoxazole) versus 68% (non-antibiotics), p >0.05.

In two patients with concordant PFGE results, susceptibility patterns differed by more than one dilution step for amoxicillin, trimethoprim and trimethoprim/sulfamethoxazole. On the other hand, differing PFGE results were associated with similar susceptibility results in eight patients.

For patients with concordant PFGE patterns, the median time between the UTI and the UTI minus 1 sample was 12 days (range 1–32). For discordant PFGE patterns, the median time was 20 days (range 5–33, p <0.05).

Discussion

In women with rUTI receiving prophylaxis, the presence of ASB was not found to be a predictor for the development of a UTI during 15 months of follow-up. Nonetheless, antimicrobial susceptibility and the PFGE pattern of asymptomatic *E. coli* strains, isolated within 1 month before an *E. coli*

TABLE 2. Positive and negative predictive values of the resistance pattern of an asymptomatic bacteriuria strain isolated in the month preceding the symptomatic urinary tract infection

	Prevalence of resistance (%)[a]	PPV[b]	95% CI	NPV[c]	95% CI
Amoxicillin	35	83	60–94	90	81–95
Amoxicillin-clavulanic acid	15	34	21–49	94	86–98
Trimethoprim	34	76	54–89	94	86–98
Trimethoprim/ sulfamethoxazole	33	76	53–89	94	86–98
Norfloxacin	14	100	96–100	97	90–99
Ciprofloxacin	13	100	96–100	97	90–99
Nitrofurantoin	0	NA[d]	NA[d]	NA[d]	NA[d]

ASB, asymptomatic bacteriuria; 95% CI, 95% confidence interval; NPV, negative predictive value; PPV, positive predictive value; UTI, urinary tract infection.
[a]Prevalence of resistance is based on susceptibility data from *Escherichia coli* strains isolated at baseline.
[b]Proportion of patients who had an asymptomatic isolate resistant to this antibiotic in whom the subsequent symptomatic isolate was also resistant to this antibiotic.
[c]Proportion of patients in whom the asymptomatic isolate was susceptible to this antibiotic and the symptomatic isolate was also susceptible to this antibiotic.
[d]Not applicable (NA) because no nitrofurantoin-resistant *E. coli* was found at baseline.

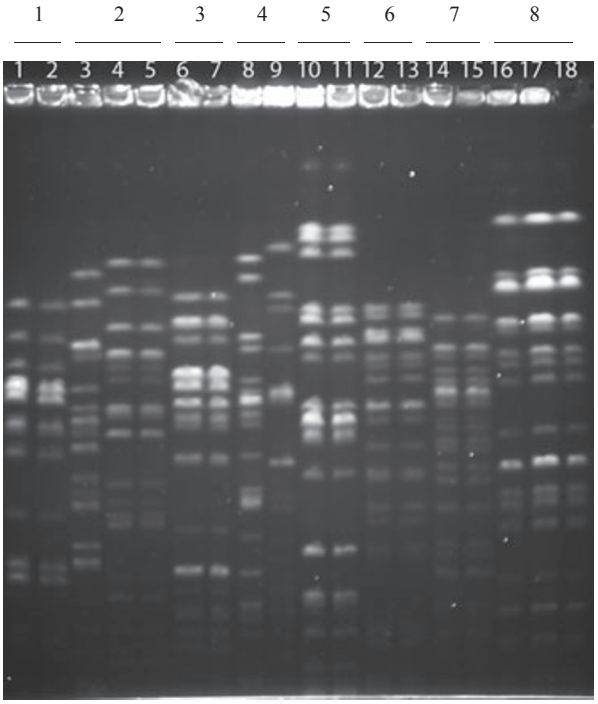

FIG. 3. Example of pulsed-field gel-electrophoresis (PFGE) patterns. The patterns of 18 *Escherichia coli* strains, obtained from eight patients, are shown. Isolates belonging to one patient are indicated by horizontal lines above the PFGE lanes. For each patient, the first lane represents the asymptomatic strain, whereas the second (and third) lane(s) represent(s) the symptomatic strain(s). Two *E. coli* pairs had different PFGE patterns (patients 2 and 4). In patients 2 and 8, PFGE was performed on two symptomatic *E. coli* strains (lanes 4 and 5, and lanes 17 and 18, respectively) with different colony morphology on the blood agar plate, but a similar PFGE pattern.

UTI, correlated well with these characteristics of the UTI-causing strain. At least 90% of the patients had a susceptible *E. coli* UTI strain when a susceptible asymptomatic *E. coli* was isolated, and this applied to all antibiotics tested. Also for resistant asymptomatic isolates high predictive values (≥75%) were found, with the exception of amoxicillin-clavulanic acid (34%). Seventy percent of the *E. coli* pairs had similar PFGE patterns.

The strength of this study was the comparison on an individual level of ASB and UTI strains with regard to antimicrobial susceptibility and PFGE pattern. Furthermore, to the best of our knowledge this was the first study that addressed the predictive value of strains causing ASB in women with rUTI. In addition, this was a sub-analysis of data that were acquired prospectively.

A limitation was that in the NAPRUTI study women did not always send a urine sample when experiencing symptoms of a UTI, so the number of microbiologically confirmed UTIs may be underestimated. However, the influence on the present analyses seems limited because using clinical recurrence as the endpoint did not alter our results. Also, our study population could be seen as heterogeneous by the inclusion of both premenopausal and postmenopausal women, with differing risk factors and clinical parameters. However, interaction testing between ASB and menopausal status revealed no significant results, indicating that the association between ASB and UTI was similar in both premenopausal and postmenopausal women. This justifies the combined analysis of these groups in the present study. Furthermore, 40% of all women had ASB at baseline, and almost all experienced an episode of ASB during the study period. Such high levels of ASB have only been observed in specific patient populations, such as catheterized patients and elderly people in long-term care facilities [19,20]. In the general population, ASB is found in 1–5% of women, and can increase to 8.6% in postmenopausal women [6,19]. The high prevalence in the present study could partly be explained by the use of a single-voided specimen to indicate ASB, instead of two consecutive sam-

ples as formulated in the original definition [20]. The use of a single specimen is now accepted as a more practical and adequate alternative [21]. However, it seems that ASB is more common in women with rUTI than in women without such a history. Finally, no placebo group was included in the NAPRUTI study, making our results mainly applicable to women with rUTI receiving prophylaxis. With long-term prophylaxis as the standard treatment for women with rUTI, this limits the external validity of our findings only marginally.

The predictive value of ASB has been previously evaluated in a prospective cohort study, which revealed a marginal association between UTI and ASB (hazard ratio 1.8, 95% CI 0.9–3.5) [9]. Another prospective study among premenopausal women showed that 8% of women with ASB developed a UTI within 1 week compared with 1% without ASB [10]. However, the women included in these prospective studies had had no history of recurrent UTI, making a comparison with our study difficult. The predictive value of ASB needs further evaluation in specific subgroups; for example, renal transplant patients, in whom ASB has been associated with a higher incidence of pyelonephritis [22].

The negative predictive value of ASB strains, in terms of antimicrobial susceptibility, was diminished after long-term trimethoprim/sulfamethoxazole prophylaxis, leading to low predictive values of ASB *E. coli* initially susceptible to amoxicillin, trimethoprim and trimethoprim/sulfamethoxazole (67%, 24% and 24%, respectively). The high prevalence of resistance to these agents was expected because their resistance genes are plasmid linked [23]. However, the clinical relevance of these low NPVs is limited because these agents will not be considered as appropriate treatment options in women receiving trimethoprim/sulfamethoxazole prophylaxis.

The value of antimicrobial susceptibility results from previously obtained isolates has also been studied by Vellinga *et al.* [16]. When the interval between two UTIs was no more than 3 months, they reported NPVs ranging from 78 to 98% and low PPVs for amoxicillin-clavulanic acid (55%) and nitrofurantoin (20%), similar to our observations. Our observed relatively low PPV for amoxicillin-clavulanic acid was mainly the result of the susceptibility breakpoint used, as most discordant *E. coli* pairs differed by only one MIC dilution step. All but one of the pairs concerned showed similar PFGE patterns, so especially in the case of amoxicillin-clavulanic acid, the use of quantitative susceptibility data is more appropriate than the susceptibility breakpoint. Vellinga *et al.* explained the low PPV of nitrofurantoin by a relatively quick decay of resistance to this agent when such resistance is found. This is in accordance with the reported low prevalence of resistance of *E. coli* to nitrofurantoin as, for example, shown by the ARESC study in which nitrofurantoin resistance did not exceed 5% [24]. The absence of resistance to nitrofurantoin observed among our *E. coli* is in line with these results. Hence, the recently recorded *E. coli* susceptibility results need to be considered in clinical practice, thereby leading to a more patient-specific approach to the treatment of women with rUTI. This can primarily be applied to patients in the hospital setting, where urinary samples are often requested by treating physicians. General practitioners can apply these findings to patients shortly after hospital discharge.

Of our asymptomatic and symptomatic *E. coli* pairs, 70% had similar PFGE patterns. A similar rate (68%) was found by Russo *et al.* [25]. During a 3-month follow-up, Czaja *et al.* [26] reported a same-strain bacteriuria of 7% 14 days before a UTI, that gradually increased to 69% 1 day before the UTI. Czaja *et al.* included women aged 18–49 years who had had a history of at least one UTI during the past year, whereas the NAPRUTI inclusion criterion was a minimum of three UTIs. The different patient characteristics of the two study populations might account for the different results [27]. We found a shorter time interval between the two sampling moments for PFGE-concordant *E. coli* strains compared with PFGE-discordant pairs, which is in accordance with the time-dependent trend reported by Czaja *et al.* [26].

Two *E. coli* pairs had similar PFGE patterns, but differed in susceptibility pattern. This might be because of the presence of integrons encoding for β-lactam, trimethoprim and sulfamethoxazole resistance, because integrons are too small to be detected by PFGE [28]. The PFGE patterns reflect more than the antimicrobial susceptibility alone and this could explain our finding that eight patients had similar antimicrobial susceptibility results but different PFGE patterns [15].

In conclusion, the presence of ASB is not predictive for the development of UTI in women with rUTI using prophylaxis, but the antimicrobial susceptibility of isolates from asymptomatic urinary samples can be used to guide antibiotic therapy when a UTI develops within 1 month.

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Transparency Declaration

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References

1. Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *Dis Mon* 2003; 49: 53–70.
2. Foxman B. Recurring urinary tract infection: incidence and risk factors. *Am J Public Health* 1990; 80: 331–333.
3. Ikaheimo R, Siitonen A, Heiskanen T *et al.* Recurrence of urinary tract infection in a primary care setting: analysis of a 1-year follow-up of 179 women. *Clin Infect Dis* 1996; 22: 91–99.
4. Hooton TM. Recurrent urinary tract infection in women. *Int J Antimicrob Agents* 2001; 17: 259–268.
5. Beerepoot MA, Ter Riet G, Nys S *et al.* Cranberries vs antibiotics to prevent urinary tract infections: a randomized double-blind noninferiority trial in premenopausal women. *Arch Intern Med* 2011; 171: 1270–1278.
6. Colgan R, Nicolle LE, McGlone A, Hooton TM. Asymptomatic bacteriuria in adults. *Am Fam Physician* 2006; 74: 985–990.
7. Mabbett AN, Ulett GC, Watts RE *et al.* Virulence properties of asymptomatic bacteriuria *Escherichia coli*. *Int J Med Microbiol* 2009; 299: 53–63.
8. Vejborg RM, Hancock V, Schembri MA, Klemm P. Comparative genomics of *Escherichia coli* strains causing urinary tract infections. *Appl Environ Microbiol* 2011; 77: 3268–3278.
9. Jackson SL, Boyko EJ, Scholes D, Abraham L, Gupta K, Fihn SD. Predictors of urinary tract infection after menopause: a prospective study. *Am J Med* 2004; 117: 903–911.
10. Hooton TM, Scholes D, Stapleton AE *et al.* A prospective study of asymptomatic bacteriuria in sexually active young women. *N Engl J Med* 2000; 343: 992–997.
11. Beerepoot MA, Nys S, Van der Wal WM *et al. Lactobacillus rhamnosus* gr-1 and l. Reuteri rc-14 versus trimethoprim-sulfamethoxazole (tmp/smx) in the prevention of recurrent urinary tract infections (rutis) in postmenopausal women: a randomized double-blind non-inferiority trial. *ICAAC* 2009. Abstract L1–1656a.
12. The European Confederation of Laboratory Medicine (ECLM). European urinalysis guidelines. *Scand J Clin Lab Invest* 2000; 60 (suppl 231): 1–96.
13. The European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society for Clinical Microbiology and Infectious Diseases (ESCMID). Eucast definitive document e.Def 3.1, June 2000: determination of minimum inhibitory concentrations (MICs) of antibacterial agents by agar dilution. *Clin Microbiol Infect* 2000; 6: 509–515.
14. Conrad S, Oethinger M, Kaifel K, Klotz G, Marre R, Kern WV. Gyra mutations in high-level fluoroquinolone-resistant clinical isolates of *Escherichia coli*. *J Antimicrob Chemother* 1996; 38: 443–455.
15. Tenover FC, Arbeit RD, Goering RV *et al.* Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995; 33: 2233–2239.
16. Vellinga A, Cormican M, Hanahoe B, Murphy AW. Predictive value of antimicrobial susceptibility from previous urinary tract infection in the treatment of re-infection. *Br J Gen Pract* 2010; 60: 511–513.
17. Freedman LS. Tables of the number of patients required in clinical trials using the logrank test. *Stat Med* 1982; 1: 121–129.
18. Schoenfeld D. The asymptotic properties of nonparametric tests for comparing survival distributions. *Biometrika* 1981; 68: 316–319.
19. Nicolle LE, Bradley S, Colgan R, Rice JC, Schaeffer A, Hooton TM. Infectious diseases society of america guidelines for the diagnosis and treatment of asymptomatic bacteriuria in adults. *Clin Infect Dis* 2005; 40: 643–654.
20. Nicolle LE. Asymptomatic bacteriuria: review and discussion of the IDSA guidelines. *Int J Antimicrob Agents* 2006; 28 (suppl 1): S42–S48.
21. Lumbiganon P, Laopaiboon M, Thinkhamrop J. Screening and treating asymptomatic bacteriuria in pregnancy. *Curr Opin Obstet Gynecol* 2010; 22: 95–99.
22. Fiorante S, Lopez-Medrano F, Lizasoain M *et al.* Systematic screening and treatment of asymptomatic bacteriuria in renal transplant recipients. *Kidney Int* 2010; 78: 774–781.
23. Kahlmeter G, Menday P. Cross-resistance and associated resistance in 2478 *Escherichia coli* isolates from the pan-European eco.Sens project surveying the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections. *J Antimicrob Chemother* 2003; 52: 128–131.
24. Naber KG, Schito G, Botto H, Palou J, Mazzei T. Surveillance study in Europe and Brazil on clinical aspects and antimicrobial resistance epidemiology in females with cystitis (ARESC): implications for empiric therapy. *Eur Urol* 2008; 54: 1164–1175.
25. Russo TA, Stapleton A, Wenderoth S, Hooton TM, Stamm WE. Chromosomal restriction fragment length polymorphism analysis of *Escherichia coli* strains causing recurrent urinary tract infections in young women. *J Infect Dis* 1995; 172: 440–445.
26. Czaja CA, Stamm WE, Stapleton AE *et al.* Prospective cohort study of microbial and inflammatory events immediately preceding *Escherichia coli* recurrent urinary tract infection in women. *J Infect Dis* 2009; 200: 528–536.
27. Zaffanello M, Malerba G, Cataldi L *et al.* Genetic risk for recurrent urinary tract infections in humans: a systematic review. *J Biomed Biotechnol* 2010; 2010: 321082.
28. Fluit AC, Schmitz FJ. Resistance integrons and super-integrons. *Clin Microbiol Infect* 2004; 10: 272–288.